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Nondestructive estimation of wood chemical composition of sections of radial wood strips by diffuse reflectance near infrared spectroscopy

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Abstract The use of calibrated near infrared (NIR) spectroscopy for predicting the chemical composition of *Pinus taeda* L. (loblolly pine) wood samples is investigated. Seventeen *P. taeda* radial strips, representing seven different sites were selected and NIR spectra were obtained from the radial longitudinal face of each strip. The spectra were obtained in 12.5 mm sections from pre-determined positions that represented juvenile wood (close to pith), transition wood (zone between juvenile and mature wood), and mature wood (close to bark). For these sections, cellulose, hemicellulose, lignin (acid soluble and insoluble), arabinan, galactan, glucan, mannan, and xylan contents were determined by standard analytical chemistry methods. Calibrations were developed for each chemical constituent using the NIR spectra, wood chemistry data and partial least squares (PLS) regression. Relationships were variable with the best results being obtained for cellulose, glucan, xylan, mannan, and lignin. Prediction errors were high and may be a consequence of the diverse origins of the samples in the test set. Further research with a larger number of samples is required to determine if prediction errors can be reduced.

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Introduction

Pinus taeda L. (loblolly pine) is the dominant plantation species in the Southeastern United States of America. The forest products industry has widely accepted the use of *P. taeda* because of its ability to grow on a wide range of sites and its suitability to produce desirable products. The deployment of genetically improved planting stock coupled with intensive silvicultural management has greatly improved growth and yields of *P. taeda* (Li et al. 1999). It is expected that the inclusion of quality traits such as stem straightness and wood quality in *P. taeda* breeding programs will greatly increase genetic gains and the value of the trees (Li et al. 1999). Unfortunately, traditional methods for measuring wood properties are generally slow, costly, and destructive, limiting their application to tree breeding programs. Thus less expensive, rapid, and nondestructive methods for measuring wood properties are required.

Recently it has been demonstrated that near infrared (NIR) spectroscopy can successfully estimate several *Pinus radiata* D. Don and *P. taeda* wood properties including air-dry density, microfibril angle (MFA), stiffness (determined using SilviScan-2 diffraction data and measured density) and a number of tracheid morphological characteristics measured by SilviScan (Evans 1997; Jones et al. 2005a, b; Schimleck and Evans 2002a, b). While the SilviScan instruments measure many wood properties at high spatial resolution, they cannot measure wood chemical composition or tracheid (or fiber) length. We have shown (Schimleck et al. 2004a) that NIR spectroscopy can be used to estimate the tracheid length of sections of *P. taeda* radial strips, but we have not attempted to use NIR spectroscopy to estimate the chemical composition of intact radial sections.

Several studies (Birkett and Gambino 1988; Garbutt et al. 1992; Michell 1995; Wright et al. 1990) have used NIR spectroscopy to estimate the chemical composition of wood but these studies did not use intact wood, rather they used milled wood generally representing a composite whole tree sample or milled increment cores. Recently Kelley et al. (2004) and Poke and Raymond (2006) estimated chemical using composition NIR spectra collected from the surface of intact wood. An advantage of working with solid wood is that it eliminates milling samples prior to NIR analysis (Poke and Raymond 2006). In other recent studies Yeh et al. (2004) and Sykes et al. (2005) used transmittance NIR spectroscopy to estimate the lignin and α-cellulose content of wood wafers sliced from the tangential surface of increment cores. Clearly if NIR spectroscopy can be used to estimate the chemical composition of radial wood sections, in addition to tracheid length, coarseness, density, MFA, stiffness, and tracheid wall thickness, then its value as a nondestructive tool for the rapid estimation of wood properties will be greatly enhanced. Therefore, the objectives of this study were to:

- Develop calibrations for wood chemical components including cellulose, hemicellulose, lignin (acid insoluble, soluble, and total) and individual

monosaccharides (arabinan, galactan, glucan, mannan, and xylan) using NIR spectra obtained in 12.5 mm sections from the radial–longitudinal surface of *P. taeda* radial wooden strips; and

- Evaluate these calibrations by estimating the wood chemical composition of sections of radial wooden strips in a separate test set based on NIR spectra obtained from the radial longitudinal face of each strip.

Materials and methods

Sample origin

Seventeen breast height discs (approximately 40 mm thick) were collected from *P. taeda* L. (loblolly pine) trees growing on seven sites of variable age and site index in GA, USA. A summary of the sites is given in Table 1. All samples were frozen and stored until processing.

While the discs were still frozen pith-to-bark radial strips (12.5 × 12.5 mm) were cut with a bandsaw from each disc for NIR and chemical analysis. The radial strips were defrosted and dried overnight in an oven set at 50°C to reduce the moisture content of the samples to approximately 7%.

Near infrared spectroscopy

Two or three 12.5 mm sections were selected on each radial strip and their positions recorded on the surface of each strip. The width of the section (12.5 mm) was used to ensure sufficient wood was available for wood chemical analysis. Sections were selected to be representative of wood close to the pith (juvenile wood), close to bark (mature wood) and the transition zone between juvenile, and mature wood. Some strips were short and insufficient wood was available to provide a section in the transition zone. NIR diffuse reflectance spectra were obtained from the sawn radial longitudinal face of each 12.5 mm section using a NIR Systems Inc. Model 5000 scanning spectrophotometer (Silver Spring, MD, USA.). Samples were held in a custom made holder

Table 1 Stand descriptions for samples used in the calibration and prediction sets including physiographic region, site index (SI_{25}), age, latitude (Lat.), and longitude (Long.)

Region	SI_{25}	Age (years)	Lat. (N)	Long. (W)
Piedmont	55	21	34°09′	85°22′
Upper Atlantic Costal Plain	78	22	31°62′	84°99′
Lower Atlantic Costal Plain	76	25	32°26′	81°27′
Lower Atlantic Costal Plain	84	25	31°11′	81°81′
Lower Atlantic Costal Plain	79	25	31°31′	81°60′
Lower Atlantic Costal Plain	80	25	31°42′	81°68′
Lower Atlantic Costal Plain	81	24	31°79′	81°57′

similar to that illustrated in Schimleck et al. (2001) with modifications to hold thicker samples. A 5×12.5 mm mask was used to ensure that a constant area was tested. Several samples were slightly twisted and a small gap between the spectrometer window and sample was occasionally observed permitting stray light to interfere with the NIR measurements. To minimize stray light the samples were tested in a light proof environment. The spectra were collected at 2 nm intervals over the wavelength range of 1,100–2,500 nm. Fifty scans were accumulated for each 12.5 mm section and the results averaged to produce one spectrum per section for analysis. The instrument reference was a ceramic standard. All measurements were made in a conditioned atmosphere maintained at 40% RH and 20°C.

Wood chemical analysis

The selected 12.5 mm sections were extracted by soaking the blocks in acetone overnight. The sections were extracted three times with the acetone being drained between extractions. Following extraction the blocks were cut into small pieces using a utility knife and ground to a powder using a small stainless steel ball mill and a Wig-L-Bug.

The extracted and ground samples were prepared for carbohydrate analysis using a methodology based upon methods described in TAPPI test method T-249 cm-00 and Pronto (1998).

The weighed samples were hydrolyzed with 72% sulfuric acid according to TAPPI T-249 cm-85, which was modified by changing the sample size and volumes. 0.175 g of air-dry sample was weighed in a digestion tube and at the same time a separate aliquot was removed for moisture analysis. After addition of 1.5 ml of 72% sulfuric acid, primary hydrolysis was carried out at 30°C for 2 h. At the end of this period the acid concentration was adjusted to 3% with the addition of water and the secondary hydrolysis carried out in an autoclave set at 121°C for 1 h. The hydrolyzed samples were cooled and filtered through tared glass fiber filters. The filtrate was collected and the volume adjusted to 50 ml. The filtrate was diluted 1–25 ml with an internal standard added to each flask.

From the diluted solution, the monosaccharide concentration was determined using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The system consisted of a Dionex HPLC system equipped with a GP50 gradient pump, and ED40 electrochemical detector, an AS40 auto sampler and a pneumatic controller for the 500 mM NaOH post-column addition solution. The column was a Dionex CarboPac PA10 $(4 \times 250$ mm) and a CarboPac PA10 guard column $(4 \times 50$ mm). Elution of the monosaccharides was achieved with a weakly basic eluent followed by a stronger base column cleanup between injections. A post column addition of base was used to enhance detection of the monosaccharides. Samples were run in duplicate.

The cellulose content (%) was determined based on the formula given by Easty and Malcolm (1982).

$$\text{Cellulose} = \text{Glucan} - (1/3 \times \text{Mannan}) \tag{1}$$

The hemicellulose content (%) was determined as the difference between the total sugars and total cellulose, i.e.,

$$\begin{aligned} \text{Hemicellulose} = {} & (\text{Arabinan} + \text{Galactan} + \text{Glucan} + \text{Mannan} + \text{Xylan}) \\ & - \text{cellulose} \end{aligned} \tag{2}$$

Acid insoluble lignin was determined from insoluble material collected on the filter after the hydrolysis procedure used for carbohydrates. The hydrolysis procedure is essentially the same as the method described in TAPPI test method T-222 om-02. Acid soluble lignin was determined using TAPPI test method, UM250. Total lignin was determined by summing acid-insoluble and acid-soluble lignin.

Calibration development

A total of 46 diffuse reflectance NIR spectra, measured from the 17 radial strips, were available for NIR analysis. In the development of preliminary calibrations it was observed that four samples had large leverage and two samples had large residual X-variance. The NIR spectra and wet chemical data of these samples were investigated. It was found that two samples had abnormal NIR spectra (those with the large residual X-variance), another two had abnormal chromatograms (the two samples having the largest leverage), while another sample had high moisture content when analyzed and suggested a weighing error. The sixth sample was excluded because of its proximity to a knot previously undetected prior to having the samples analyzed.

Owing to the small number of samples (40) preliminary calibrations were developed using all samples. Following the development of calibrations using

Table 2 Summary of variation in measured wood chemical properties for the calibration and prediction sets

Constituent	Calibration set (28 spectra)				Prediction set (12 spectra)			
	Minimum (%)	Maximum (%)	Avg. (%)	SD	Minimum (%)	Maximum (%)	Avg. (%)	SD
Arabinan	0.97	1.64	1.27	0.17	0.94	1.52	1.21	0.19
Cellulose	32.27	40.19	36.17	2.36	31.91	39.24	35.59	2.29
Galactan	1.20	6.29	2.21	1.29	1.48	3.73	2.39	0.80
Glucan	34.94	44.03	39.44	2.59	34.53	42.98	38.98	2.61
Hemicellulose	20.60	26.77	23.11	1.43	21.47	25.27	23.44	1.18
Mannan	8.01	12.40	9.81	1.08	7.85	11.68	10.19	1.20
Xylan	5.20	8.21	6.54	0.78	5.50	7.43	6.26	0.70
Insoluble lignin	25.91	30.82	27.92	1.20	25.84	30.86	27.97	1.67
Soluble lignin	0.31	0.40	0.36	0.03	0.30	0.39	0.35	0.03
Total lignin	26.25	31.19	28.28	1.22	26.23	31.18	28.38	1.73

all samples the spectra were split at random into calibration (28 spectra, representing 12 radial strips) and prediction (12 spectra, representing 5 radial strips each from a different site) sets. A statistical summary of the calibration and prediction sets is given in Table 2.

Calibrations were developed using partial least squares (PLS) regression with four cross validation segments and a maximum of ten factors (Unscrambler, version 8.0, Camo AS, Norway). The final numbers of factors used for each calibration were those recommended by the Unscrambler software. Calibrations were developed using second derivative spectra, left and right gap widths of 8 nm were used for the conversion, limiting the wavelength range available for calibration development to 1,108–2,492 nm. The standard error of cross validation (SECV) (determined from the residuals of each cross validation phase), the standard error of calibration (SEC) (determined from the residuals of the final calibration), and the co-efficient of determination (R^2) were used to assess calibration performance.

The standard error of prediction (SEP) was used to give a measure of how well a calibration predicts the parameter of interest for a set of unknown samples that are different from the calibration set. The predictive ability of calibrations was assessed by calculating the ratio of performance to deviation (RPD) (ratio of the standard deviation of the reference data to the SEP) (Williams and Sobering 1993). An RPD of greater than 2.5 is considered satisfactory for screening (Williams and Sobering 1993).

Results and discussion

Variation in NIR spectra

Second derivative diffuse reflectance NIR spectra of two *P. taeda* wood samples (one juvenile wood the other mature wood) that show wide variation in their chemical composition are shown in Fig. 1. For the 2 spectra shown glucan content varied from 34.53 to 44.03% and total lignin varied from 26.25 to 31.18%.

When the juvenile and mature wood NIR spectra are compared large differences between the two types of wood can be observed. The juvenile wood spectrum has a lower overall absorbance relative to the mature wood spectrum because the mature wood sample has greater density giving higher absorbance. Many bands in the NIR spectrum from 1,100–2,500 nm have been assigned to cellulose (1,490, 1,780, 1,820, 1,900, 1,930, 2,100, 2,276, 2,336, 2,352, and 2,488 nm) while others (1,143, 1,417, 1,446,1,668, 1,685, and 2,132 nm) have aromatic origins (Osborne et al. 1993; Shenk et al. 1992). For the samples utilized in this study Table 3 lists the three wavelengths that have the strongest relationship with each property.
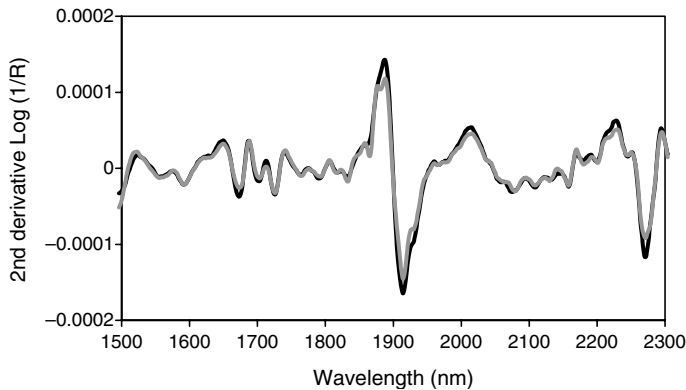
Fig. 1 Second derivative NIR spectra for juvenile wood (*grey line*) and mature wood (*black line*)

PLS calibrations—full set

Forty NIR diffuse reflectance spectra, obtained from the radial longitudinal face of *P. taeda* radial strips, were available for calibration development. Calibrations obtained for each chemical component are summarized in Table 4.

Table 3 Summary of the three wavelengths that give the strongest relationship per component, the R^2 value for each wavelength is presented beside the wavelength in parentheses

Constituent	1st wavelength (nm)	2nd wavelength (nm)	3rd wavelength (nm)
Arabinan	1,780 (0.60)	1,778 (0.56)	1,228 (0.56)
Cellulose	1,610 (0.49)	1,608 (0.41)	1,452 (0.39)
Galactan	1,130 (0.34)	1,156 (0.32)	1,606 (0.30)
Glucan	1,610 (0.53)	1,608 (0.44)	2,178 (0.42)
Hemicellulose	2,306 (0.40)	1,732 (0.38)	2,308 (0.37)
Mannan	1,130 (0.55)	1,696 (0.54)	1,698 (0.53)
Xylan	1,232 (0.66)	1,694 (0.65)	1,230 (0.65)
Insoluble lignin	1,132 (0.51)	1,130 (0.50)	1,714 (0.48)
Soluble lignin	1,294 (0.51)	1,312 (0.52)	1,292 (0.51)
Total lignin	1,130 (0.51)	1,132 (0.51)	1,704 (0.49)

Table 4 Summary of calibrations developed using all 40 samples

Constituent (%)	No. of factors	R^2	SEC	SECV
Arabinan	3	0.72	0.09	0.12
Cellulose	5	0.84	0.92	1.68
Galactan	1	0.18	1.04	1.11
Glucan	4	0.80	1.12	1.78
Hemicellulose	3	0.54	0.91	1.13
Mannan	5	0.93	0.30	0.64
Xylan	6	0.95	0.16	0.41
Insoluble lignin	5	0.88	0.47	1.02
Soluble lignin	2	0.57	0.02	0.02
Total lignin	5	0.88	0.48	1.03

Note soluble lignin and therefore total lignin was not available for one sample

Cellulose, glucan, mannan, xylan, insoluble, and total lignin displayed strong calibration coefficients with R^2 greater than 0.80. Of these many are strongly related to each other, such as glucan and cellulose (R-value = 0.99) and total lignin and insoluble lignin (R-value = 0.99). The calibration for arabinan had an R^2 of 0.72 while the relationships for acid-soluble lignin (0.57), hemicellulose (0.54), and galactan (0.18) were weak to poor. Figure 2 shows the relationships between measured values and NIR-estimated values for arabinan (a), cellulose (b), mannan (c), and total lignin (d).

For almost all calibrations the SECV, which is considered to be a better measure of calibration error than the SEC, was considerably larger than the SEC. A high SECV indicates that the sample excluded in each cross validation phase was not well predicted by the corresponding calibration and that the SEC is overly optimistic. The large difference between the SEC and the SECV may be a consequence of the small number of trees included for each site and the large number of sites represented (7). It is probable that if a larger calibration set was used then the SEC and SECV would be more similar.
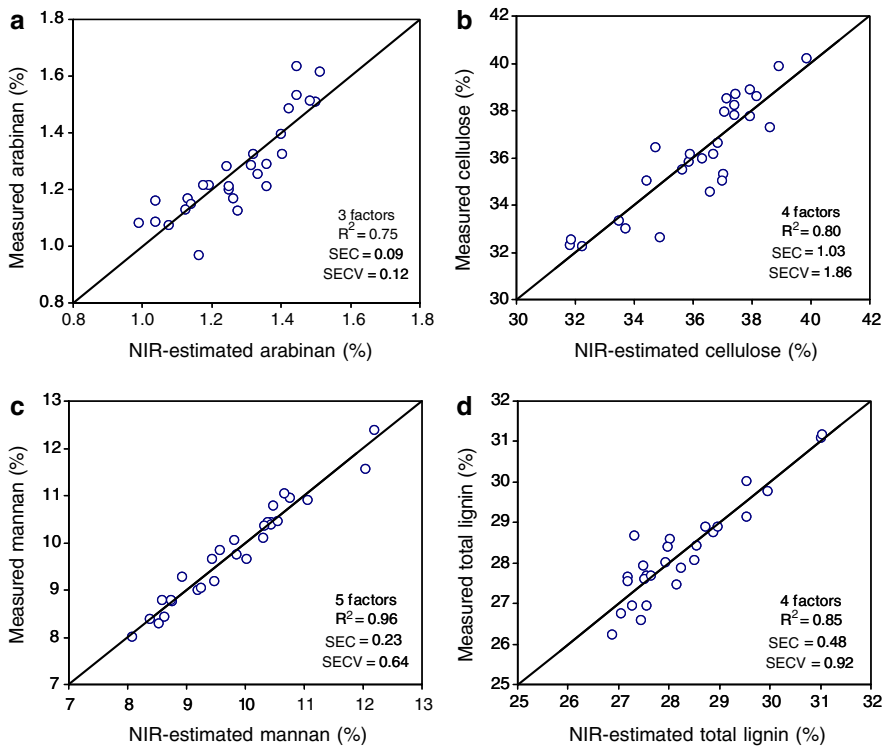

Fig. 2 Relationships between analytical chemistry and NIR-estimated values for arabinan (**a**), cellulose (**b**), mannan (**c**), and total lignin (**d**). Note that the regression line has been plotted in all figures

Table 5 Summary of calibrations developed using 28 samples and applied to the test set of 12 samples

Constituent (%)	Calibration set (28 samples)				Prediction set (12 samples)		
	No. of factors	R^2	SEC	SECV	R_{p}^2	SEP	RPD
Arabinan	3	0.75	0.09	0.12	0.68	0.11	1.73
Cellulose	4	0.80	1.03	1.86	0.57	1.73	1.32
Galactan	1	0.25	1.11	1.23	0.11	0.97	0.83
Glucan	4	0.82	1.09	1.96	0.57	1.88	1.39
Hemicellulose	3	0.59	0.92	1.24	0.30	1.04	1.14
Mannan	5	0.96	0.23	0.64	0.66	0.71	1.71
Xylan	6	0.98	0.11	0.44	0.61	0.46	1.52
*Insoluble lignin	4	0.85	0.47	0.91	0.51	1.26	1.33
Soluble lignin	2	0.66	0.02	0.02	0.36	0.02	1.50
*Total lignin	4	0.85	0.48	0.92	0.51	1.21	1.43

*Note: one sample was omitted from the insoluble and total lignin calibrations

Prediction of wood chemical composition

The set of 40 spectra were split into a calibration (28 samples) and prediction set (12 samples). No samples from the five trees in the prediction set were present in the calibration set but the sites of the trees were represented. Table 5 provides a summary of the calibrations and their performance on the prediction set. A prediction R^2 (R_{p}^2) was calculated as the proportion of variation in the independent prediction set that was explained by the calibration.

The calibration statistics were similar to those reported in Table 3 with cellulose, insoluble and total lignin, glucan, mannan, and xylan all having strong relationships, but with each having a SECV much larger than the SEC.

When the calibrations were applied to the prediction set, R_{p}^2 were noticeably lower than the corresponding R^2, while SEP and SECV were similar. Figure 3 shows the relationships between measured values and NIR-predicted values for arabinan (a), cellulose (b), mannan (c), and total lignin (d). The strongest R_{p}^2 (0.68 and 0.66) were obtained for the predictions of arabinan and mannan. The remaining constituents noted for their good calibration statistics gave R_{p}^2 ranging from 0.11 to 0.61.

Discussion

The calibration statistics obtained using both sets (40 and 28 samples) suggest that NIR spectroscopy may provide a useful tool for the rapid assessment of lignin and monosaccharide contents of intact sections of radial longitudinal strips though predictive statistics must be improved for the properties examined in this study to be estimated on a routine basis. The predictive performance of the calibrations, based on the subset of 28 samples, may have suffered because of the diverse origins of the samples in the test set. Other research based on *P. taeda* and using NIR spectroscopy has been successful in
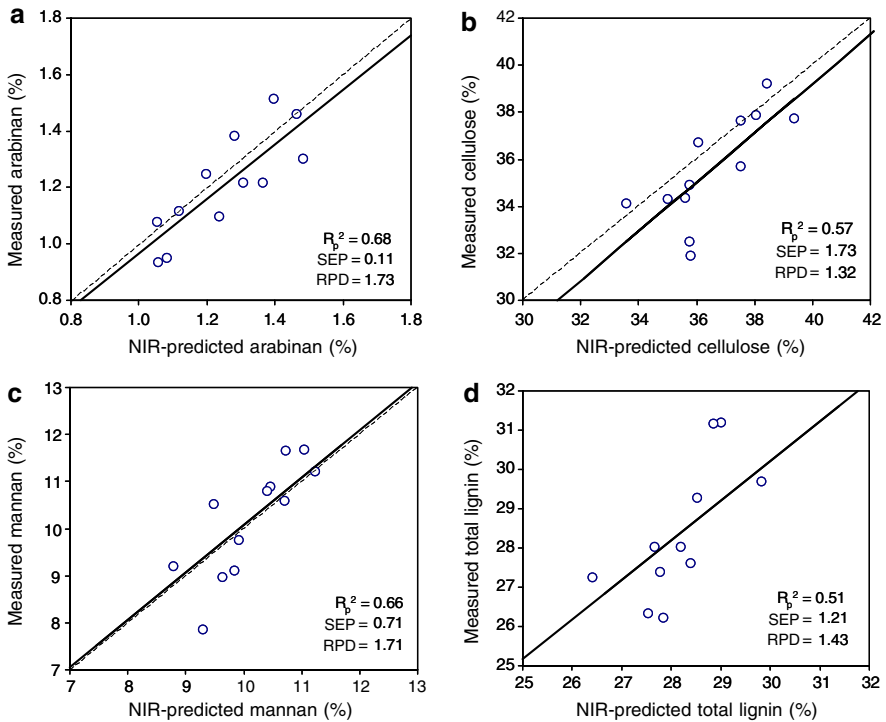
Fig. 3 Relationships between analytical chemistry and NIR-predicted values for arabinan (**a**), cellulose (**b**), mannan (**c**), and total lignin (**d**). Note that the regression line has been plotted (*thick, dark line*) in all figures and that the *thin broken line* indicates a one-one relationship between measured and predicted values

predicting the morphological and physical properties of samples from different sites, in this work a large number of samples from various sites in Georgia were included in the calibration set and the predictive success of the calibrations was attributed to the diversity represented (Jones et al. 2005a, b). Hence if a larger calibration set was utilized in this study, stronger predictions may have been obtained. Further research with larger sample sets is required to develop calibrations and apply them to estimate wood chemical composition. However, the high cost associated with standard analytical chemical analyses limits the number of samples that can be examined. The time and cost of analytical chemical methods emphasizes the importance of developing a rapid alternative method to routinely measure cellulose, glucan and lignin contents on a large-scale.

The current cost of wet chemistry is both an argument for using NIR spectroscopy and the limiting factor in creating calibrations with a large enough population. This highlights the importance of identifying suitable samples prior to wood chemical content analysis. Win ISI, a software that uses a neighborhood concept to identify spectrally unique samples that best represent the population, was successfully used with *Eucalyptus nitens*, where

estimated genetic gains in cellulose content, based on NIR estimates from a subset of samples were similar to those obtained when the full sample set was analyzed using wet chemistry (Schimleck et al. 2004b). Perhaps a similar approach could work with pine species.

The results reported in this study demonstrate that it is possible to calibrate reflectance NIR spectroscopy for the estimation of glucan, xylan, cellulose and lignin contents with intact radial strips of wood. The work presented in this study complements that of Jones et al. (2005a, b) and Schimleck et al. (2004a) who established the utility of using NIR spectroscopy for estimating air-dry density, MFA, stiffness, and a number of tracheid characteristics; including coarseness, length, and wall thickness of radial strips. The ability of NIR spectroscopy to estimate a wide range of properties, including several not measured by SilviScan, makes NIR spectroscopy a valuable tool for wood property assessment.

Conclusions

This study demonstrates that calibrations can be created for lignin and monosaccharide content of intact *P. taeda* radial strips. Prediction errors were high and may be a consequence of the diverse origins of the samples in the test set. Further research with a larger number of samples is required to determine if prediction errors can be reduced.

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